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Association between fluoride exposure in drinking water and cognitive deficits in children: A pilot study



Tewodros Rango Godebo^{a,*}, Marc Jeuland^b, Redda Tekle-Haimanot^c, Biniyam Alemayehu^c, Arti Shankar^d, Amy Wolfe^e, Nati Phan^a

^a Department of Environmental Health Sciences, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA

^b Sanford School of Public Policy and Duke Global Health Institute, Duke University, Durham, NC, 27708, USA

^c Addis Ababa University, School of Medicine, Department of Neurology, Addis Ababa, Ethiopia

^d Department of Biostatistics and Data Science, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA

^e University of Kentucky, Kentucky Geological Survey, Lexington, KY, 40506, USA

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ABSTRACT

Fluoride (F) exposure in drinking water may lead to reduced cognitive function among children; however, findings largely remain inconclusive. In this pilot study, we examined associations between a range of chronic F exposures (low to high: 0.4 to 15.5 mg/L) in drinking water and cognition in school-aged children (5–14 years, n = 74) in rural Ethiopia. Fluoride exposure was determined from samples of community-based drinking water wells and urine. Cognitive performance was measured using: 1) assessments of ability to draw familiar objects (donkey, house, and person), and 2) a validated Cambridge Neuropsychological Test Automated Battery's (CANTAB) Paired Associate Learning (PAL), which examines memory and new learning and is closely associated with hippocampus function of the brain. Associations between F^- and cognitive outcomes were evaluated using regression analysis, adjusting for demographic, health status, and other covariates. The median (range) of water and urine F^- levels was 7.6 (0.4–15.5 mg/L) and 6.3 (0.5–15.7 mg/L), respectively; these measures were strongly correlated (r = 0.74), indicating that water is the primary source of F⁻ exposure. Fluoride in drinking water was negatively associated with cognitive function, measured by both drawing and CANTAB test performance. Inverse relationships were also found between \overline{F} and drawing objects task scores, after adjusting for covariates (p < 0.05). Further analysis using CANTAB PAL tasks in the children confirmed that F⁻ level in drinking water was positively associated with the number of errors made by children (p < 0.01), also after adjusting for covariates (p < 0.05). This association between water F⁻ and total errors made became markedly stronger as PAL task difficulty increased. Fluoride exposure was also inversely associated with other PAL tasks-the number of patterns reached, first attempt memory score and mean errors to success. These findings provide supportive evidence that high F⁻ exposures may be associated with cognitive deficits in children. Additional well-designed studies are critically needed to establish the neurotoxicity of F⁻ in children and adults exposed to both low levels known to protect dental caries, as well as excess F⁻ levels in drinking water.

1. Introduction

Worldwide, millions of people are affected by fluorosis due to the consumption of drinking water containing levels of fluoride (F^-) that exceed the WHO recommended level of 1.5 mg/L (WHO, 2000). An optimal amount of F^- (0.7-1 mg/L) is well-recognized as essential for preventing dental caries (O'Mullane et al., 2016; Medjedovic et al., 2015; U.S. DHSS-FP [U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation], 2015; US Centers for

Disease Control and Prevention (US CDC), 1999), but excessive intake of F^- from sources such as water, food and F^- -containing dental products can lead to dental and skeletal fluorosis (Rango et al., 2020, 2017, 2014; Ayoob and Gupta, 2006). In recent years, F^- exposure has received additional scrutiny due to findings linking F^- exposure with potential cognitive effects, such as a reduced intelligence quotient (IQ) in children (Goodman et al., 2022; Grandjean, 2019; Das and Monda, 2016; Grandjean and Landrigan, 2014; Choi et al., 2012; Tang et al., 2008; US NRC, 2006; however, other studies have not found similar associations

* Corresponding author. E-mail address: tgodebo@tulane.edu (T.R. Godebo).

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(Aggeborn and Oehman, 2021; Barberio et al., 2017; Broadbent et al., 2015).

Experimental studies on rodents provide one source of evidence for potential effects of F on the brain. These studies have shown that F crosses the placenta and blood brain barriers (Niu et al., 2018; Sharma et al., 2017; Agency for Toxic Substances and Disease Registry (ATSDR), 2003; Mullenix et al., 1995; Ron et al., 1986) and F related histopathological changes in various brain regions, including the hippocampus (critical for learning and memory), the prefrontal cortex (executive function), and the cerebellum (motor control) (Ge et al., 2018; Lee et al., 2016; Liu et al., 2014; Jiang et al., 2014a; Whitford et al., 2009; Shivarajashankara et al., 2002; Mullenix et al., 1995). Fluoride can also impair the function of myelin and neurotransmitters, increase lipid peroxidation, and inhibit several key neuronal enzymes, suggesting potential direct impairments to brain function (Niu et al., 2018; Shivarajashankara et al., 2002; Jiang et al., 2014b). The neurotoxic effect of high levels of F⁻ exposure is frequently referenced to the hippocampus, which is involved in learning, memory, and attention (Bittencourt et al., 2023; Grandjean, 2019 and reference therein; Pereira et al., 2011; Valdez-Jimenez et al., 2011, Bhatnagar et al., 2002; Mullenix et al., 1995). Given that F⁻ readily crosses the placenta (Sharma et al., 2017; Agency for Toxic Substances and Disease Registry (ATSDR), 2003), high exposures have been shown to damage the developing brain in utero, leading to permanent long-term effects (Yan et al., 2016; Grandjean and Landrigan, 2014). This is particularly relevant in F endemic rural regions that have limited alternative water sources and where exposures are potentially chronic, spanning from conception to adulthood. Several epidemiological studies, for example from Asian countries, have shown an association between higher drinking water exposures in F⁻ endemic regions and reduced IQ (Dong et al., 2018; Karimzade et al., 2014; Choi et al., 2012), as well as other cognitive effects on children such as inattentive behaviors (Bashash et al., 2018, 2017).

The Main Ethiopian Rift (MER) Valley is one of the best-known endemic regions for chronic exposures to low to elevated F^- in community-based groundwater sources. Extensive studies in the region have documented the adverse health effects—dental and skeletal fluorosis—of chronic exposures to elevated F^- (e.g., Rango et al., 2020, 2012, 2013; Tekle-Haimanot, 1990). Most children in the region were born and raised in a single location, in- and out-migration in the region is low, and F^- exposures therefore typically occur over a lifetime starting at conception, but varying considerably in concentration, depending on the water source being used. Prior research in the Rift Valley has not examined effects of chronic F^- exposures on children's cognition.

To address this knowledge gap, we investigated the relationship between exposures to F from drinking water and children's performance in several cognitive tasks. The first such task involved drawing of familiar objects and animals, which are considered valid and reliable instruments that correlate with general intelligence in children (Yong, 2015; Imuta et al., 2013; Ebersbach and Hagedorn, 2011; Brook, 2009; Reynolds, 1978). For example, research using the Wechsler Intelligence Scale for Children has shown a moderate correlation between drawing ability and intelligence (Imuta et al., 2013; Reynolds and Hickman, 2004; Abell et al., 1996, 2001). Ebersbach and Hagedorn (2011) showed that spatial drawing ability was positively related to cognitive flexibility in 7 to 11 years old children. Successful drawing requires cognitive flexibility, which develops only gradually with age (Jolley, 2008). Second, we examined the association of F⁻ exposure in drinking water with scores on the standardized cognitive tasks of the Cambridge Neuropsychological Test Automated Battery (CANTAB®, Cambridge Cognition Ltd., UK) of the Paired Associates Learning (PAL). In doing so, the study also provided an assessment of the feasibility of administering the CANTAB neurodevelopmental tests to children in a rural African setting. These tests evaluate visual memory and new learning, for which the hippocampus of the brain region is critical.

In this study, we enrolled children exposed to naturally occurring

and wide-ranging drinking water concentrations of $F^-(0.41 \text{ to } 15.5 \text{ mg/} \text{L})$ since conception. We tested the hypothesis that chronic F^- exposure in drinking water is associated with a child's ability to draw familiar objects of varying difficulty (a donkey, a house and a person) and cognitive performance using a standardized CANTAB task. The children residing in sample communities come from a homogeneous rural population that generally engages in farming for its livelihood and has common living conditions, culture, and diet. Such similarity within a study population is rare in epidemiological studies, and dissimilar to the situation in studies in industrialized countries, where socioeconomic conditions vary substantially, and populations face much lower and less-variable F^- exposures. Thus, this location presents an ideal setting to study the health effects of wide range of chronic F^- exposures.

2. Materials and methods

2.1. Study population

In this cross-sectional study, we enrolled 74 children in the MER, aged 5-14 years, in two sampling periods between 2019 and 2021. In previous work, we mapped the distribution of F⁻ levels in several drinking water sources, including community-based wells in use in the study area. This work revealed that elevated levels of F⁻ due to deposition of volcanic aquifer sediments enriched with this element are widespread (Rango et al., 2013, 2012, 2010). Working from the prior mapping, 8 communities were selected to cover a wide range of F levels in drinking water sources that spanned from 0.41 to 15.5 mg/L (Rango et al., 2019, 2013, 2012). All enrolled children were confirmed to have been born and raised in sample villages that used these respective wells, and thus were known to have been chronically exposed to relatively stable F⁻ concentrations since conception. We assume that current concentrations are a reliable proxy for "historical" concentrations within the study area, which is consistent with our understanding of the hydrogeochemistry of the region and the consistent use of these sources (which have been in place for 15-65 years across the sample villages) in each study community.

Notably, the population of these rural farming communities is characterized by similar genetic origin (i.e., ethnicity), education, dietary patterns, cultural and social values, livelihoods activities, and many behaviors (e.g., low rates of smoking, and rare if any use of toothpaste), but vary by F^- exposure. The relative uniformity of these parameters helps to minimize the risk of confounding of F⁻ exposures by other factors that influence health. Study participants live in households that are engaged in cereal-based agriculture as their primary livelihood activity and are generally low in income and wealth. We recruited children at community centers that are typically located near each community well. Study inclusion criteria were: Consent to participate (obtained from both parents and children), permanent residence in the community, age between 5 and 14 years, and duration of residency that was at least as long as the age of the sampled well from which drinking water was being consumed. In each community, though we did not explicitly stratify by age and sex, we did attempt to select individuals to obtain a relatively good distribution of different ages and sex.

Survey data were then collected with each enrolled respondent, to record sex, age, place of birth, exposure to smoking, toothpaste usage, approximate daily water intake, and anemic appearance. For the latter, a trained neurologist looked for clinical signs of anemia, specifically pallor on conjunctiva, brittle or spoon-shaped fingernails, tongue redness and swelling, and presence of headache symptoms. The recorded estimate of daily water intake (in liters, L), weight (kg) and the water F^- concentrations (mg/L) were used to calculate the daily F^- intake dose (mg/kg/ day), as the product of water F^- concentration (mg/L) and water intake (L)), divided by the body weight of each child. We also recorded weight and height using an electronic scale for weight and measuring tapes for height. These measures were used to calculate the body mass index (BMI) for each study participant (BMI: weight(kg)/height(m²)) as a

proxy for nutrition status.

The study received ethical approval from the Institutional Review Board (IRB) at Tulane University (Protocol No. 2018–043) and locally from the National Research Ethics Review Committee (NRERC; reference no. MoSHE/144/1096/19). All participants provided consent, and parents/guardians gave permission for children to participate in addition to children giving their own assent.

2.2. Measurements of exposure

2.2.1. Sampling of drinking water and urine

We collected a total of 68 urine samples (24-h urine (n = 46) and spot urine (n = 22) samples) from children residing in 8 community-based wells (Fig. S1). These 8 wells had F⁻ concentrations ranging between 0.41 and 15.5 mg/L. All sample collection materials for water and urine were pre-cleaned with 1 N HNO₃ and 1 N HCl and rinsed three times with deionized water. Water samples were then filtered in the field directly into 60 mL polyethylene bottles using luer lock syringes and 0.45 µm Mixed Cellulose Ester membrane filters. Urine samples were collected in disposable plastic urine collection containers with a closing cap (each with a capacity of 1 gal). The volume of each collected urine sample was registered, and the sample was immediately transferred into a 60 mL polyethylene bottle. Participants were shown how to avoid contamination and instructed to carefully collect urine samples. Water and urine samples were then kept in a zero-degree freezer, and properly packed, stored, and transported to the lab at Tulane University, USA.

2.2.2. Fluoride, As, and Pb analysis in drinking water and urine

Fluoride content in water and urine was determined using the Ion Selective Electrode (ISE), buffering the standards and samples using equal volume ratios with a total ionic strength adjustment buffer (TISAB II). The water and urine sample concentrations of As and Pb, which are known to also affect cognition, were also measured using an Inductively Coupled Plasma-Mass Spectrometer (Agilent 7900 ICP-MS) at Tulane lab. The recovery for F⁻, As, and Pb in samples with respect to the NIST SRM 2668 low standard was between 90 and 110%. In a recent and related biomonitoring study (Rango et al., 2019), we observed a significant positive correlation between F⁻ and As in drinking water and urine, highlighting the role of drinking water as the main route of exposure to both of these elements. Two (5.2 and 15.5 mg/L of F) of the eight community wells have only been used intermittently in the most recent period, because of well pumping malfunctions that were also observed during sampling. Given that urinary levels of these elements only reflect recent exposures that are not from these sources, we exclude them from the analysis of associations between urinary \boldsymbol{F}^- and cognition.

2.2.3. Urine correction for urine dilution

Analyses of F⁻, As, and Pb concentrations in urine samples with a volume \leq 300 mL (n = 22), which can be considered spot samples rather than 24-h samples, were adjusted for specific gravity (SG) in order to account for variations in urine dilution. This enhances comparability of results from these two types of urine samples. Strong correlations (r = 0.78, p < 0.0001) have been reported between SG-adjusted spot urine sample and fluoride in a 24-h urine sample (Zohouri et al., 2006), indicating that adjustment for urinary dilution approximates a 24-h biomarker. To apply this adjustment, SG was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD) that was calibrated with deionized water before each measurement. The refractometer prism head was rinsed in deionized water after each reading. Urinary F⁻ levels were normalized for dilution by SG adjustment using the following formula (MacPherson et al., 2018; Hauser et al., 2004).

 $F_{SG} = F \times \left[(SG_M - 1) / (SG - 1) \right]$

where: F_{SG} is the SG–corrected F⁻ concentration (mg/L).

F is the observed F^- concentration (mg/L), and.

 SG_{M} is the median specific gravity for the study cohort (SG_{M} = 1.012).

2.3. Measurements of outcomes

2.3.1. Children's drawing tasks

A total of 68 (37 males and 31 females) from the 74 children were enrolled and asked to participate in three drawing tasks of common objects that children readily encounter or experience in the study area, though their reproduction varies in complexity: a house, a person, and a donkey. They were provided with a pencil, rubber eraser, drawing pad, table, chair and allowed as much time to draw as they needed to complete their drawings, but no instruction or support was provided other than the name of the items to be produced. All children submitted drawings of each object, such that a total of 204 drawings were collected and scored. Most children took approximately 20-30 min to finish all three drawings. We developed scoring criteria based on the completeness of each object such that a point was given for each part correctly drawn (Table 1). Each object also received an additional score for overall appearance ranging from 0 to 4 (bad (0), poor (1), fair (2), good (3), very good (4)). Similar figure drawing criteria were used in other studies to assess child cognition (Panesi and Morra, 2016; Imuta et al., 2013). All drawings were independently scored by two examiners who were blinded to the F⁻ concentrations in well water; the inter-rater reliability was assessed and showed a strong correlation (r > 0.92).

2.3.2. Cambridge neuropsychological test automated battery (CANTAB)

To further examine the association between F⁻ in water and cognitive outcomes, additional standardized tests were conducted using the Cambridge Neuropsychological Test Automated Battery (CANTAB®, Cambridge Cognition Ltd., UK). For this study, we selected one of the CANTAB's tests, Paired Associate Leaning (PAL), which is most sensitive to spatial memory and learning and linked to the medial temporal lobe (e.g., hippocampus). This is the brain region most thought to be affected by F⁻ toxicity (Mullenix et al., 1995). The test administrator was trained in CANTAB, described the CANTAB instructions and demonstrated to each child how the iPad touch screen works using local language (Amharic or Oromegna). CANTAB is language and culture neutral and is a computerized test administered on a touch-screen interface that requires very little language comprehension, making it suitable for use with children (Luciana and Nelson, 2002; Fray and Robbins, 1996). Prior to initiating the main tests, a prescreening test with the Motor Screening Task (MOT) was administered to introduce participants to CANTAB and provide a general assessment of sensorimotor, vision, movement, or comprehension difficulties. For the MOT task, a series of crosses that appeared in random locations on the screen was presented to the child. The examiner first demonstrated the correct touching procedure using the forefinger of the dominant hand to touch the cross,

Table 1

Scoring criteria developed	l to assess the qualit	y of each o	drawn object.
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Criteria score (parts scale + overall appearance)				
A person (11 scale)	A donkey (8 scale)	A house (6 scale)		
Two hands (1)	Four legs (1)	Cross on the top (1)		
Two legs (1)	Head (1)	Roof (1)		
Head (1)	Ears (1)	Grass cover (1)		
Fingers (hand+foot) (1)	Eyes (1)	Door (1)		
Ears (1)	Mouth (1)	Two windows (1)		
Nose (1)	Hair (1)	Wall (1)		
Eyes (1)	Tail (1)			
Mouth (1)	Neck (1)			
Hair (1)				
Neck (1)				
Shoulder (1)				
Overall appearance (0 to 4)	Overall appearance (0 to 4)	Overall appearance (0 to 4)		

score is better (Table S1).

and the child then completed the test. The CANTAB PAL task also begins with two rounds of practice sessions. The examiner performed the first practice round, and the participant performed the second. The task then moved to the assessment phase, which had four levels: 2, 4, 6 and 8 stimuli over the trials. No enrolled children had difficulties completing the tasks.

The PAL test assesses visual memory and new learning that depends on spatial planning ability. In this test, boxes are opened in random order on the screen to reveal their contents. The patterns inside the boxes are then displayed in the middle of the screen one at a time, and the participants must touch the box in which the pattern was originally located. The level of difficulty increases during the test, with 2-, 4-, 6-, and 8-pattern stages. The primary outcomes include the *total errors adjusted* (accounting for the number of trials completed), where a lower number is better, and the *number of patterns reached*, where a higher

2.3.3. Statistical analysis

Demographic, anthropometric, F^- concentrations, drawing scores and PAL measures (errors/stages completed) were first described by their quartiles and means \pm standard deviation (Tables 2 and 3). The $F^$ level in water was analyzed as a continuous variable and categorized into three F^- exposure groups (in mg/L) (Group 1; reference low $F^$ group): <3, Group 2: >3–8, and Group 3: >8–15.5 mg/L). Comparisons of means in the different F^- exposure groups, and by sex were carried out with one-way ANOVA. We then utilized a linear regression model to examine the associations between F^- exposure and the drawing scores for the three objects (a donkey, a person, a house), and CANTAB PAL tasks, adjusting for As and Pb in drinking water, sex, grade levels, BMI, and anemic appearance, and tested water F^- and PAL difficulty

Table 2

Statistical descriptions of anthropometric values, measured concentrations of F⁻, As, and Pb from 8 groundwater wells, demographic, and lifestyle factors of the children who performed the drawing tasks.

			Percentiles				
	N	Min	25th	50th	75th	Max	$\text{Mean} \pm \text{SD}$
Anthropometric measures							
Age	68	5	8	10	12	14	10.0 ± 2.44
Weight (kg)	68	16.5	22.9	25.3	33.1	48.2	28.2 ± 7.45
Height (m)	68	1.04	1.24	1.31	1.47	1.59	1.34 ± 0.14
BMI (kg/m^2)	68	12.3	14.3	15.2	16.2	21.1	15.4 ± 1.57
Children's grade level	68	0	1.0	1.0	3.0	6.0	1.97 ± 1.44
Biomarkers of F ⁻ Exposures							
Water F ⁻ , As, and Pb concentrations							
Individuals water intake (liter/day)	68	0.3	0.9	0.9	1.13	1.5	0.91 ± 0.23
Individuals F intake (mg/day)	68	0.123	3.22	6.48	9.59	23.3	7.24 ± 4.93
Individuals Dose (mg/kg bw/day)	68	0.005	0.124	0.23	0.35	1.15	0.27 ± 0.21
F^{-} in community wells (mg/L)	8	0.41	3.47	7.6	10.7	15.5	7.55 ± 4.79
As in community wells (ug/L)	8	0.92	2.57	5.41	10.8	21.9	7.3 ± 6.83
Pb in community wells $(\mu g/L)$	8	0.001	0.01	0.1	0.44	0.73	0.23 ± 0.27
Urinary F ⁻ . As, and Pb concentrations							
F^{-} in urine (mg/L)	48	0.54	3.54	6.34	91	15.7	644 + 40
As in urine (ug/I)	47	1.46	5.02	6.77	15.8	49.1	12 ± 12
Ph in urine (μ_g/L)	47	0.2	0.61	0.95	1 23	5 76	12 ± 12 1.05 ± 0.84
A ge distribution by F^- exposure groups	17	0.2	0.01	0.90	1.20	0.70	1.00 ± 0.01
	17	7	8	10	11.5	14	10 ± 2.12
3-8 mg/I	25	5	75	9	12	13	99 ± 2.12
>8-15 5 mg/I	25	6	8	10	12 12 3	13	10.2 ± 2.0
$V_{0} = 13.0 \text{ mg/ L}$	20	0	0	10	12.5	14	10.2 ± 2.0
<2 mg/l	17	0	1	1	3 5	6	1.99 ± 1.76
< 3 mg/L	25	0	1	1	3.5	5	1.00 ± 1.70 1.04 ± 1.42
> 9 15 5 mg/L	23	0	1	2	2.05	5	1.64 ± 1.43
>6-15.5 lllg/L Weight by F exposure groups	20	0	0	3	5.25	5	2.13 ± 1.78
2 mg/l	17	20.6	22.0	24.7	22.2	49.0	202 1 7 02
< 3 llg/L	17	20.0 16 E	23.0	24.7	32.3	40.2	20.3 ± 7.02
> 9.15.5 mg/L	23	10.5	22.9	23	33	42.7	27.7 ± 0.91
>8-15.5 lllg/L	20	18.2	20.8	27.1	34.4	44.4	28.0 ± 7.85
Height by F exposure groups	17	1.00	1.07	1 01		1 54	1 05 1 0 10
<3 mg/L	17	1.22	1.27	1.31	1.41	1.56	1.35 ± 0.10
>3-8 mg/L	25	1.16	1.23	1.3	1.43	1.56	1.33 ± 0.12
>8–15.5 mg/L	26	1.04	1.22	1.33	1.49	1.59	1.35 ± 0.16
BMI by F exposure groups			. –				
<3 mg/L	17	13	17	15.4	16.3	18.5	15.4 ± 1.4
>3-8 mg/L	25	12.3	14.5	15.5	15.9	19	15.3 ± 1.4
>8–15.5 mg/L	26	13	17	15.4	16.3	18.5	15.4 ± 1.4
Sex distribution by F exposure groups							
<3 mg/L ($n = 17$): Male/Female	9/8						
>3-8 mg/L ($n = 25$): Male/Female	12/13						
>8–15.5 mg/L (<i>n</i> = 26): Male/Female	11/15						
Lifestyle Factors	n						
Anemic appearance – present	33(48.5%)						
Anemic appearance – absent	35(51.5%)						
Current smoking habit- present	0 (0%)						
Current smoking habit – absent	68 (100%)						
Current toothpaste use - present	12(17.6%)						
Current toothpaste use – absent	56(82.4%)						

Note: The urinary F⁻, As, and Pb concentrations of individuals from two of the eight community wells were excluded because the tested well water was only used intermittently in those settings and may not accurately represent current exposure.

Table 3

Statistical descriptions of concentrations of F⁻ from 8 groundwater wells, and children's drawing scores and CANTAB PAL task performance scores.

NMin25th50th75thMaxMean \pm SDBIOMARKER OF F ⁻ EFFECT (drawing performance)Donkey drawing total scores at F ⁻ exposure groups0.41-15.5 mg/L680.51.547.7511.5 4.76 ± 3.39 $< 3 mg/L$ 1713.55.59.7511 6.18 ± 3.43 $> 3-8 mg/L$ 250.51.547.511.5 4.64 ± 3.47 $> 8-15.5 mg/L$ 260.513.25611 3.96 ± 3.10 Person drawing total scores at F ⁻ exposure groups0.41-15.5 mg/L680.54.6381113.57.88 ± 3.63 $< 3 mg/L$ 170.558.51213.58.59 ± 3.46 $> 3-8 mg/L$ 250.55911.312.37.98 ± 3.63 $< 3 mg/L$ 250.55911.312.37.98 ± 3.63 $> 3-8 mg/L$ 250.55911.312.37.98 ± 3.63
BIOMARKER OF F EFFECT (drawing performance) Donkey drawing total scores at F exposure groups 0.41-15.5 mg/L 68 0.5 1.5 4 7.75 11.5 4.76 ± 3.39 $< 3 mg/L$ 17 1 3.5 5.5 9.75 11 6.18 ± 3.43 $> 3 \cdot 8 mg/L$ 25 0.5 1.5 4 7.5 11.5 4.64 ± 3.47 $> 8 - 15.5 mg/L$ 26 0.5 1 3.25 6 11 3.96 ± 3.10 Person drawing total scores at F^- exposure groups 0.41-15.5 mg/L 68 0.5 4.63 8 11 13.5 7.88 ± 3.63 $< 3 mg/L$ 17 0.5 5 8.5 12 13.5 8.59 ± 3.46 $> 3 \cdot 8 mg/L$ 25 0.5 5 9 11.3 12.3 7.98 ± 3.46 $> 3 \cdot 8 mg/L$ 26 0.5 5 9 11.3 12.3 7.98 ± 3.46
Donkey drawing total scores at F^- exposure groups 68 0.5 1.5 4 7.75 11.5 4.76 \pm 3.39 $< 3 mg/L$ 17 1 3.5 5.5 9.75 11 6.18 \pm 3.43 $> 3 \cdot 8 mg/L$ 25 0.5 1.5 4 7.5 11.5 4.64 \pm 3.47 $> 8 \cdot -15.5 mg/L$ 26 0.5 1 3.25 6 11 3.96 \pm 3.10 Person drawing total scores at F^- exposure groups 68 0.5 4.63 8 11 13.5 7.88 \pm 3.63 <3 mg/L 17 0.5 5 9.9 11.3 12.3 7.98 \pm 3.63 $< 3 mg/L$ 17 0.5 5 9 11.3 12.3 7.98 \pm 3.63 $< 3 mg/L$ 25 0.5 5 9 11.3 12.3 7.98 \pm 3.46 $> 8 \cdot 15 \cdot m m^2$ 26 0.5 5 9 11.3 12.3 7.98 \pm 3.46
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Person drawing total scores at F ⁻ exposure groups 68 0.5 4.63 8 11 13.5 7.88 ± 3.63 <3 mg/L
$<3 \text{ mg/L}$ 170.558.51213.58.59 \pm 3.46 $>3.8 \text{ mg/L}$ 250.55911.312.37.98 \pm 3.46 $> 0.15 \text{ France}$ 260.55911.312.37.98 \pm 3.46
$>3-8 \text{ mg/L}$ 25 0.5 5 9 11.3 12.3 7.98 \pm 3.46
$>6-15.5 \text{ mg/L}$ 20 0.5 5 8 11 13.5 7.33 \pm 3.78
House drawing total scores at F^- exposure groups
0.41–15.5 mg/L 68 0.5 3.63 5.5 7.0 10.0 5.2 ± 2.47
$<\!\!3 {\rm mg/L} \qquad 17 \qquad 1 \qquad 5 \qquad 6.0 \qquad 7.25 \qquad 10 \qquad 6.12 \pm 2.28$
$>3-8$ mg/L 25 0.5 3.75 5.0 6 8.5 4.88 ± 2.10
$>8-15.5 \text{ mg/L}$ 26 0.5 2.63 5.25 7 9.0 4.92 ± 2.83
BIOMARKER OF F^- EFFECT (CANTAB PAL performance)
PALTEA (PAL Total Errors (Adjusted))
0.41–15.5 mg/L 74 5 18 33.5 47.5 69 34.1 ± 18.8
$<3 mg/L$ 20 5 13 18.5 34 62 24.0 \pm 16.7
$>3-8 mg/L$ 26 9 20.8 34 48 68 35.7 ± 18.1
$>8-15.5$ mg/L 28 10 21.5 41 56.8 69 39.7 ± 18.6
PALNPR (PAL Number of Patterns Reached)
0.41–15.5 mg/L 74 2 6 8 8 8 6.6 ± 1.89
<3 mg/L 20 4 8 8 8 7.4 ± 1.31
$>3-8 mg/L$ 26 2 6 8 8 8 6.46 ± 2.1
$>8-15.5 mg/L$ 28 2 4 6 8 8 6.14 ± 1.96
PALMETS (PAL Mean Errors to Success)
0.41–15.5 mg/L 69 0 0 2 3.5 6 2.04 ± 1.69
$<3 mg/L$ 20 0 1.25 2 4 5 2.4 \pm 1.43
$>3-8 mg/L$ 23 0 0 2 4 6 2.1 \pm 1.95
$>8-15.5 mg/L$ 26 0 0 2 3 5 1.73 ± 1.64
PALFAMS (PAL First Attempt Memory Score)
0.41–15.5 mg/L 74 0 4.75 8 12 17 8.15 ± 4.54
$<3 mg/L$ 20 3 8.25 11.5 14 15 10.5 \pm 3.68
$>3-8 mg/L$ 26 0 4.75 7.5 11.3 17 7.46 \pm 4.50
$>8-15.5 mg/L$ 28 0 2.25 7.5 10.8 15 7.11 \pm 4.67

Note that PALTEA = PAL Total Errors (Adjusted); PALNPR = PAL Number of Patterns Reached; PALFAMS = PAL First Attempt Memory Score; PALMETS = PAL Mean Errors to Success.

interactions on PAL outcomes. Results are presented as regression coefficients β with their 95% confidence intervals (95% *CIs*), allowing for clustering of standard errors at the community level owing to the withincommunity correlation of exposures as well as other independent variables. Study hypotheses were evaluated at the 5% level of significance. All analyses, summaries, and graphs were performed using the Statistical Analysis System (SAS 9.4, SAS Institute, Cary, NC, and GraphPad Prism 9.3.1).

3. Results

3.1. General characteristics

The average age of children in the sample was 10 years; approximately half (54.4%; n = 37) of these were male (Table 2). Based on the WHO (2000) classification of BMI, most children would be categorized as underweight with the 75th percentile falling below 16.2 kg/m², and a mean BMI of 15.4 \pm 1.57 kg/m². The F⁻ concentrations in the groundwater and urine samples ranged between 0.41 and 15.5 mg/L and 0.54 and 15.7 mg/L, respectively. All the water samples except that with 0.41 mg/L exceeded the 1.5 mg/L standard limit in drinking water (WHO, 2017). The interquartile ranges of estimated daily F⁻ intake per body weight per day, and urinary F⁻ concentration were 3.22–9.59 mg/day, 0.124–0.35 mg/kg bw/day and 4.02–4.65 mg/

L, respectively. Most of the children (88.2%; n = 60) therefore ingested an estimated daily amount of F⁻ that exceeds the U.S. EPA's No-Observed-Adverse-Effects-Level (NOAEL) value of 0.06 mg/kg/day value for F⁻ (US Environmental Protection Agency (U.S. EPA), 2002). Educational and health services, and dietary patterns were generally homogeneous across sample areas. Young children are usually breast fed for up to 2 years, then weaned onto household foods, and the diet is primarily cereal-based (maize, wheat, teff), with meat consumption being rare (Rango et al., 2012). Arsenic concentrations in groundwater ranged between 0.92 and 22 µg/L, and 2 of the 8 wells (11.8 and 22 µg/L) exceeded the established standards of 10 µg/L for As concentration in drinking water (WHO, 2017). The Pb concentrations in the groundwater were all below 1 µg/L, and none of the water samples exceeded the 10 µg/L limit established for Pb in drinking water (WHO, 2017; US EPA, 2001).

3.2. Association of F^{-} As, and Pb in drinking water and urine

We observed strong correlation between F^- in drinking water and children's urine (r = 0.74, p < 0.001). This strongly suggests that drinking water is the main source of F^- for the children. Moderate correlations were observed between As and Pb in drinking water and children's urine—(r = 0.33, p = 0.023) and (r = 0.28, p = 0.054), respectively. A positive and moderate association was also found



Fig. 1. Linear regression plots showing the association between F⁻ in drinking water and children's (5 to 14 years old; n = 68) drawing ability scores for: A) a donkey ($r^2 = 0.075$, p = 0.024); B) a house ($r^2 = 0.023$, p = 0.21), and a person (not shown) ($r^2 = 0.015$, p = 0.32). C) integrating all objects (a donkey, a house and a person) drawing scores ($r^2 = 0.027$, p = 0.019).

between F⁻ and As in drinking water (r = 0.23, p = 0.057). Conversely, a negative association was found between F⁻ and Pb in the drinking water samples (r = -0.61, p < 0.001). The unique geochemistry of groundwater in the MER (i.e., the alkaline pH > 8, and oxidizing aquifer conditions) limits the occurrence of Pb and other toxic heavy metals such as Cd and Ni, and results in a very low concentration of these elements in groundwater of the study region (Rango et al., 2019, 2013). Other than

As, the concentrations of heavy metals are generally low in the Rift groundwater and expected to have minimal role on cognition.

3.3. Association of F^- in drinking water with drawing performance

The distribution of the scores for figure drawing (based on completed parts and overall appearance as shown in Table 1), in the three F^- exposure groups were as follows (Table 3): 1) <3 mg/L communities: 6.2 (donkey), 8.6 (person) and 6.1 (house), 2) >3-8 mg/L communities: 4.6 (donkey), 8.0 (person), 4.9 (house), and 3) >8–15.5 mg/L communities: 4.0 (donkey), 7.3 (person), and 4.9 (house).

Regression analyses confirm the inverse associations between F concentrations in water and children's drawing scores (Fig. 1). In continuous analysis (Table 4), the correlation between F⁻ in drinking water and the scores for drawings of a donkey is statistically significant with a β of -0.2 points (95% CI: -0.37, -0.03) that explains 7.5% of the total variance in scores (Table 4A, Fig. 1A). After adjusting for other factors (sex, children's grade levels, BMI, As and Pb in drinking water, anemic appearance), this association weakens slightly but remains significant. Representative children's drawings of a donkey for children in different F exposure groups are shown in Fig. 2. Similarly, inverse relationships were found between water concentrations of F⁻ and drawing scores for a house (Table 4B, Fig. 1B, Fig. S2), and person (Table 4C), but these relationships were not statistically significant. Fluoride in drinking water accounted for a relatively smaller percentage of the variance in scores for these two tasks-2.4% and 1.5%, respectively.

Integrating scores for all three objects, a significant inverse relationship was found between F^- in water and drawing scores (Fig. 1C). In a sensitivity analysis, with grade level replaced by age among the covariates, the association follows the same trend with a mildly lower effect size (Table S2).

In unadjusted categorical analysis (Table 5), the F⁻ water concentration category indicators explain 7.0 and 5% of the variability in donkey and house drawing scores. While all object drawing scores decrease with higher F⁻ concentrations (Table 3), the drawing scores for the donkey task were significantly lower in the highest exposure Group 3 (>8–15.5 mg/L), compared to the lowest reference F⁻ exposure Group 1 (<3 mg/L). The adjusted β for this comparison is -3.56 points (95% *CI*: -6.62, -0.48). In contrast, though scores were also lower in Group 2 (>3-8 mg/L), the association was not significant. Similarly, for the person and house drawing tasks, children in the two higher exposure groups (>3-8 mg/L, and >8-15.5 mg/L) had lower scores than those in the reference group, but this relationship was not statistically significant. Across F exposure groups, males had a better drawing ability (particularly for the donkey and person tasks) than females, though this association between scores and sex did not reach statistical significance (Fig. S3).

3.4. Association of F^{-} in urine with drawing performance

We observed inverse associations between the F⁻ concentrations in urine and the drawing ability of children (a donkey, a house, and a person). Children's scores for the donkey drawing again showed the strongest inverse correlation with F⁻ in urine (p = 0.12) with a β of -0.19 and 95% *CI* (-0.43, 0.05) that explains 5% of the variance (Fig. 3). Inverse relationships are also observed between urinary F⁻ and drawing scores for a person ($r^2 = 0.03$, p = 0.24) and a house ($r^2 = 0.01$, p = 0.49), similarly to the drinking water analysis; however, these relationships are not significant.

3.5. Association between exposure to F^- and children's CANTAB PAL tasks

Out of the six PAL tasks that children completed (Table S1), scores for four (PALTEA, PALNPR, PALMETS, and PALFAMS) are associated

Table 4

			5-14 years old			
		Unadjusted			Adjusted	
	β (95%CI)	R ²	p-value	β ^a	<i>p</i> -value	
a. Donkey drawing					$R^2 = 0.39$	
Water F	-0.2 (-0.37, -0.03)	0.075	0.024	-0.21	0.030	
b. House drawing					$R^2 = 0.44$	
Water F	-0.082 (-0.21, 0.05)	0.024	0.21	-0.003	0.96	
c. Person drawing					$R^2 = 0.34$	
Water F	-0.09 (-0.28, 0.09)	0.015	0.32	-0.15	0.13	

Abbreviation: β , regression coefficient; *CI*, confidence interval.

^a Adjusted for sex, grade level, BMI, As and Pb in drinking water and anemic appearance.

with F⁻ concentrations in drinking water (Table 6). Increased F⁻ in drinking water is significantly associated with the number of PAL total errors adjusted (PALTEA) made by the children ($\beta = 1.2, 95\%$ CI: 0.32, 2.1; Fig. 4A). Fluoride in drinking water is also inversely correlated with the PAL number of patterns reached (PALNPR) ($\beta = -0.1$, 95%CI: -0.19, -0.01; Fig. 4B) and the number of times the correct box is chosen on first attempt (PALFAMS) when recalling pattern location ($\beta =$ -0.21 95%CI: -0.42, 0.01); p = 0.06). Finally, the mean number of attempts needed to successfully complete a stage (PALMETS) is also negatively correlated with F⁻ concentration ($\beta = -0.075$, 95%CI: 0.16, 0.007); p = 0.07). When controlling for covariates (children's grade level, BMI, As and Pb in drinking water, anemic appearance), however, only the PALTEA association with F⁻ concentration remains significant. In a sensitivity analysis, with grade level replaced by age among the covariates, the association follows the same trend with a mildly lower effect size (Table S3). Similar trends were observed in unadjusted and adjusted relationships between F in urine and CANTAB PAL tasks for PALTEA, PALNPR, and PALFAMS. In unadjusted and adjusted categorical analysis (Table S4), significant differences were found between the lowest (<3 mg/L) and highest (<8-15.5 mg/L) drinking water concentration groups, for the PALTEA, PALNPR and PALFAMS tasks.

3.6. Association between exposure to F^- and the difficulty of CANTAB PALTEA tasks

Fluoride concentration in drinking water may have larger effects as

the level of task difficulty (i.e., the number of boxes) increases. The highest average number of errors by the children were measured in the most difficult task (8-box) among those children exposed to the highest F^- in water (i.e., >8–15.5 mg/L) (Fig. 5). The lowest number of errors were made for the lowest task difficulty (2-box) among those exposed to <3 mg/L of F^- . There was no statistically significant interaction between F^- in drinking water and the task difficulty (number of PALTEA boxes) on the total errors made by the children (Fig. 5).

4. Discussion and conclusion

In this study, we assessed the association between chronic exposure to naturally-occurring F^- in drinking water and cognitive function in school-aged children, as measured using two distinct types of assessments: a simple drawing task of familiar objects, and the CANTAB PAL tests. The sample was recruited from 8 communities primary exposed to chronic F^- ranging from 0.41 to 15.5 mg/L in the MER. These communities have relatively homogenous populations with similar lifestyles and stable residency, but the residents of different villages use community-based drinking water sources that vary in their F^- levels. We hypothesized that measures of cognitive performance would decline with exposure to elevated F^- concentrations. Accordingly, we found adverse associations of F^- exposures in drinking water with children's drawing and CANTAB task performance. The strongest and most significant negative impacts were observed for the more challenging drawing task—a donkey (Fig. 1A). It is observed that children struggled



Fig. 2. Representative children's drawings of a donkey for children in different F^- exposure groups. The figures were selected by averaging the scores at each community and picked a drawing close to the mean score. Note that the label on each figure represents water concentration of F^- (mg/L) / drawing score.

Table 5

Associations between F⁻ concentration groups in drinking water and children's object drawing scores.

Fluoride concentration		Relative object drawing scores					
In drinking water (mg/L)	Crude, β (95% <i>CI</i>)	P value	Adjusted, ^a β (95% CI)	P value			
For donkey							
Water F exposure group	$R^2 = 0.07$		$R^2 = 0.40$				
Group 1 (< 3)	Reference		Reference				
Group 2 (>3–8)	-1.54 (-3.6, 0.55)	0.07	-2.88(-5.7, -0.08)	0.29			
Group 3 (>8–15.5)	-2.2 (-4.3, -0.17)	0.03	-3.56 (-6.62, -0.48)	0.024			
For house							
Water F exposure group	$R^2 = 0.05$		$R^2 = 0.46$				
Group 1 (< 3)	Reference		Reference				
Group 2 (>3–8)	-1.24 (-2.77, 0.30)	0.11	-0.46 (-2.46, 1.54)	0.64			
Group 3 (>8–15.5)	-1.21 (-2.74, 0.31)	0.21	-0.26 (-2.44, 1.91)	0.81			
For person							
Water F ⁻ exposure group	$R^2 = 0.02$		$R^2 = 0.35$				
Group 1 (< 3)	Reference		Reference				
Group 2 (>3–8)	-0.60 (-2.90 , 1.68)	0.59	-1.53 (-4.7, 1.65)	0.33			
Group 3 (>8–15.5)	-1.28 (-3.55, 0.99)	0.26	-2.77 (-6.23, 0.69)	0.11			

 β , regression coefficient; *CI*, confidence interval.

^a Adjusted for sex, children's grade level, BMI, As and Pb in drinking water, anemic appearance.



Fig. 3. Linear regression plots showing the association between F^- in urine and children's (5 to 14 years old; n = 48) drawing scores in the donkey task ($r^2 = 0.052$, p = 0.12).

more when drawing a donkey than a house or a person, which may be indicative of a greater challenge accessing memory for this task. In contrast, children appeared to have an easier time drawing a person or a house, and associations between drawing performance and F⁻ exposures were correspondingly weaker. Consistent with the negative associations between drawing skill and F⁻ exposure, children drinking from wells in communities with higher F⁻ levels performed worse in CANTAB PAL tasks that are used to test new learning and memory, and especially the PAL total errors adjusted measure. It was also observed that higher F⁻ levels were related to higher deficits in the more difficult PALTEA tasks (i.e., increasing number of boxes from 2 to 8) (Fig. 5). The PAL test targets hippocampal function by measuring visual memory and new learning (Barnett et al., 2016; de Rover et al., 2011). A study by Choi et al. (2015) found that measured working memory using the Wechsler Intelligence Scale for Children-Revised (WISC-IV) in children was negatively associated with dental fluorosis (a marker of early life F exposure during critical periods of tooth development, the first 8 years) and Wechsler's total and backward digit span tests. Goodman et al. (2022) also reported that visual-spatial and perceptual reasoning abilities may be more impacted by F⁻ exposure as compared to verbal abilities.

In previous related studies, drawing (e.g., a person) has often been used as a nonverbal screening measure of cognitive ability that may indicate visual sensory input and neuromuscular output (Imuta et al.,

Table 6

Associations between F⁻ concentrations in drinking water and children's performance in CANTAB PAL tasks.

		5-14 years old					
	Uı	Unadjusted			Adjusted		
	β (95%CI)	\mathbb{R}^2	p-value	^a β (95%CI)	p-value		
a. PALTEA				$R^2 = 0.12$			
Water F ⁻	1.2 (0.32, 2.1)	0.093	0.008	1.32 (0.05, 2.6)	0.05		
b. PALNPR				$R^2 = 0.10$			
Water F	-0.1 (-0.19, -0.01)	0.064	0.03	-0.1 (-0.22, 0.014)	0.11		
c. PALFAMS (PAL First Attempt Memory Score)				$R^2 = 0.08$			
Water F	-0.21 (-0.42, 0.01)	0.048	0.06	-0.19 (-0.51, 0.13)	0.23		
d. PALMETS				$R^2 = 0.10$			
Water F	-0.075 (-0.16, 0.007)	0.047	0.07	-0.016 (-0.13, 0.09)	0.78		

 β , regression coefficient; *CI*, confidence interval.

^a Adjusted for children's grade level, BMI, sex, As and Pb in drinking water, anemic appearance. Note that PALTEA = PAL Total Errors (Adjusted); PALNPR = PAL Number of Patterns Reached; PALFAMS = PAL First Attempt Memory Score; PALMETS = PAL Mean Errors to Success.



Fig. 4. Linear regression plots showing the association between F^- in drinking water and A) children's PALTEA (r = 0.30, p = 0.008), and B) PALNPR (r = 0.25, p = 0.03) performance.



Fig. 5. Linear regression plots showing the effect of increasing F^- in drinking water on mean CANTAB PAL total errors made by the children for tasks featuring different numbers of PAL boxes. Note: The interaction of F^- and PAL difficulty levels was not significantly related to the mean PAL total errors made by the sample children (p = 0.085).

2013; Revnolds and Hickman, 2004; Kamphaus and Pleiss, 1991; Kamphaus and Pleiss, 1991; Reynolds and Hickman, 2004; Abell et al., 1996, 2001). A study by Panesi and Morra (2016) assessed dog drawing in relation to executive function and working memory and found that these two parameters jointly accounted for 58.3% of the variance of dog drawing skill. Moreover, working memory individually accounted for the largest variance (15.4%), whereas executive function accounted for 4.4%. The interaction of these two predictors was then responsible for the remaining 38.5% of this joint variance. Evidence of the role of working memory and executive function in drawing flexibility was also reported by Morra, 1994 and Barlow et al., 2003. Moreover, environmental factors can impede these aspects of cognition and drawing. For example, a study by Guillette et al. (1998) observed impairments in memory, social interaction, creativity, drawing ability, and motor skills in a population of Mexican children exposed to pesticides relative to a comparable group living in an unexposed area. Most dramatically, pesticide exposed children scored more poorly in a "draw a person" task, which may indicate lower cognitive ability or poor visuomotor coordination.

In our study, grade level (or age) was positively associated with drawing ability, which is also consistent with prior literature (Panesi and Morra, 2016). Owing to our sampling approach, however, which aimed to balance sex and age within and across communities, the children in each community are similar, such that sex- and age-related effects cannot explain the variation in observed outcomes across communities. Other possible confounders include As and Pb, which are known to be neurotoxic contaminants, but these were found at low levels in drinking water from the sample communities and in samples of children's urine. The concentrations of As and Pb in drinking water ranged between 0.92 and 21.9 μ g/L (mean:7.3 \pm 6.83 μ g/L), and 0.001 to 0.73 μ g/L (mean: 0.23 ± 0.27 µg/L), respectively. Anemic appearance, as diagnosed from clinical signs of anemia (e.g., pallor on conjunctiva), was observed in 45.5% of the children, and is known to impair motor and mental development in infants, children, and adolescents (Lam and Lawlis, 2017; Burden et al., 2007; Lazoff, 2007). In regression analysis the association of anemic appearance, and As and Pb in water and urine did not significantly correlate with performance measures, however, this exploratory study relied on a relatively small sample and used cross sectional data to proxy for long-term exposure. In addition, the purposeful recruitment of children to obtain representative age and sex distributions around specific community wells limits the representativeness of the sample. As a result, the study may not be viewed as providing a definitive analysis of F⁻'s neurotoxicity in children. Nonetheless, the similar sociodemographic and lifestyles in these communities minimizes the risk of confounding by variables that may be correlated with exposures and cognitive performance measures. An important additional limitation was the small number of sample communities and wells. In particular, when adjusting the standard errors for clustering within wells/communities, the statistical significance of the association between water F⁻ and the PALTEA task performance scores was reduced from p = 0.034 to p = 0.09, emphasizing the need to increase the number of wells and study participants to obtain greater statistical power.

Other limitations include a lack of control of parental variables such as maternal age, educational level of parent, socio-economic status, and assessment of chemical mixture models for better exposure and effect characterization other potential neurotoxicants (e.g., As, Pb), and elemental deficiencies such as iodine and iron that may modify cognition (Lam and Lawlis, 2017). For some urine biomarker measures that were collected as spot samples, we accounted for dilution using urine SG, to reflect actual F^- exposure from drinking water.

5. Conclusion

Our findings suggest that there are cognitive impairments among children exposed to higher F^- concentrations, evaluated using figure

drawing performance and validated CANTAB cognitive tools. This study also successfully demonstrated the use of language and culture neutral CANTAB testing in a rural Ethiopian sample of children for the first time. Thus, CANTAB can feasibly be administered in this and other similar rural African contexts (as also shown by Chetty-Mhlanga et al., 2022, Chetty-Mhlanga et al., 2018; Nkhoma et al., 2013). While this exploratory study adds evidence and concern about the potential neurotoxicity of elevated F⁻ exposure, more studies are critically needed to better establish neurodevelopmental impacts of a range of F⁻ exposures from gestation to adulthood, using rigorous study designs and advanced methodologies including mixture models for exposure and effect characterization. Such studies would help provide concrete evidence to inform leaders and policy makers on the need for effective approaches to mitigate environmental exposures to F⁻, including in F⁻ endemic geographic settings such as the study areas where alternative water sources are limited, or to establish the threshold levels at which such exposures become toxic, and specifically, inform the growing controversy over the safety of water fluoridation.

Authors contributions

Tewodros Rango Godebo: Design of study concept, conducted the field work, administered the figure drawing and CANTAB cognitive assessment, data analysis and interpretation, and wrote the manuscript.

Marc Jeuland, Arti Shankar, and Amy Wolfe: Statistical data analysis, and critical revision of the manuscript for important intellectual content.

Nati Phan: Figure drawing assessment, and critical revision of the manuscript for important intellectual content.

Tekle-Haimanot, and Biniyam Ayele: Field work assistance, and revision of the manuscript for important intellectual content.

Declaration of Competing Interest

The authors declare they have no actual or potential competing interests.

Data availability

Data will be made available on reasonable request.

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Appendix A. Supplementary data

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